Cardiac Iron Deposition in Idiopathic Hemochromatosis: Histologic and Analytic Assessment of 14 Hearts From Autopsy

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In each heart taken from autopsies of 14 men with idiopathic hemochromatosis, the conduction system, atria and 10 sites in the ventricles were histologically graded for stainable iron. Stainable iron was exclusively sarcoplasmic; none was observed in the interstitium. The histologic grade for the same anatomic site varied among hearts and among different anatomic sites in the same heart. Ten hearts had stainable iron in all ventricular sites; one of the three hearts from patients who had undergone therapeutic phlebotomy had no iron at any site. Seven hearts had iron in the atria but at a lesser grade than that found in the ventricles; six hearts had mild focal iron deposition in the atrioventricular conduction system. None of the 14 hearts had stainable iron in the sinus node.

Elemental iron was quantitated by atomic absorption spectroscopy in ventricular specimens contiguous to those studied histologically and also in age-matched control hearts. Elemental iron content was markedly increased in hearts with idiopathic hemochromatosis compared with control hearts ($p < 0.01$). The quantity of elemental iron varied greatly, similar to stainable iron, but was highest subepicardially. Among the hearts from the 11 patients without prior phlebotomy, three had *no* stainable iron in the right ventricular septal subendocardium, suggesting that sampling error may be a problem in the evaluation of hemochromatosis by endomyocardial biopsy.

The sarcoplasmic location of the iron indicates that cardiac involvement in idiopathic hemochromatosis represents a storage disease and not an infiltrative process; this finding is consistent with the normal ventricular wall thicknesses observed.

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Idiopathic hemochromatosis is an autosomal recessive disorder, characterized by parenchymal iron loading of indeterminate cause, that may lead to multiple organ failure in untreated patients (1). HLA genotyping studies (2) and autopsy studies (3,4) suggest that this disorder is more common than previously recognized. Its incidence, once believed to be 1/10,000, has recently been estimated to be 2 to 3/1,000, and 10% of the population may be heterozygous (2).

Cardiac failure is the leading cause of death in idiopathic hemochromatosis (1). However, the cardiac histopathologic abnormalities in this disease have not been well described. Previous reports (5–8) on the cardiac histopathology of iron overload disorders have been confined almost exclusively to patients with acquired hemochromatosis. The purpose of this study was to describe the microscopic features and to analyze elemental iron in the heart from autopsy of patients with idiopathic hemochromatosis.

Methods

Study cases. Fourteen hearts from the tissue registry at our institution were studied. These were from patients with idiopathic hemochromatosis who died between 1947 and 1983; diagnosis was antemortem in seven and postmortem in seven. The criteria for inclusion in the study were postmortem demonstration of hepatic iron deposition and associated cirrhosis without a history of anemia and transfusion or known excessive oral ingestion of iron. For each patient with idiopathic hemochromatosis, the clinical history was reviewed and the age, sex and cause of death were recorded.

The 14 hearts were weighed, and their wall thicknesses were measured. Each heart was also evaluated grossly for the presence of valvular and ischemic heart disease. Only grade 4 coronary atherosclerosis (>75% obstruction of a

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cross-sectional area) was considered a critical lesion. In addition, 14 hearts without evidence of disease, matched with the idiopathic hemochromatosis hearts for age of patients at death and duration of formalin preservation, were obtained as controls for iron analyses.

Histology. Histologic estimations of stainable iron and fibrosis in the 14 study hearts were made by two of the investigators without knowledge of the results of the iron analyses. The extents of both stainable iron and fibrosis were graded by using an arbitrary 5 point scale (0 = 0%; 1 = 1 to 25%; 2 = 26 to 50%; 3 = 51 to 75%; 4 = >75%) based on the number of myocytes containing stainable iron or of myocardium involved by fibrosis. The anatomic sites studied included the right ventricular free wall; the right and left ventricular septal subendocardium; a core specimen from the septum; specimens from the subendocardial, midmyocardial and subepicardial layers of the left ventricular anterior and inferior walls at the level of the mitral papillary muscles; the atria; the sinus node and the atrioventricular conduction system.

Analysis of ventricular elemental iron. Dried ventricular specimens contiguous to those studied histologically (total of 140 specimens) were analyzed for elemental iron by flameless atomic absorption spectroscopy (Perkin-Elmer model 500). Specimens from each of the 10 ventricular sites in the 14 control hearts also were studied. Quality control studies, using National Bureau of Standards reference material, showed agreement within 1%.

Statistical analysis. For the elemental iron determinations, matched pair analysis was performed to test for the significance of differences between idiopathic hemochromatosis and control hearts by anatomic site—paired t test for continuous variables and signed-rank test for ordinal variables. Repeated measures of analysis of variance for continuous variables and Friedman's procedure for ordinal variables were used to determine whether differences in levels of myocardial iron by anatomic site in individual hearts reached statistical significance. Student-Neuman-Keuls multiple comparison procedures were used to determine which sites were different from one another. Spearman's rank correlation coefficient was used to estimate the degree of association between methods of estimation of elemental iron; $p < 0.05$ was regarded as evidence of statistical significance.

Results

Age and sex distribution and cause of death. All of the 14 patients and 14 control subjects were men. Age ranges were 43 to 74 years for idiopathic hemochromatosis and 44 to 75 years for controls (mean age for each group = 59 years). Causes of death in the idiopathic hemochromatosis group were liver failure in seven patients, hepatocellular carcinoma in three, sudden death in two, sepsis in one and acute aortic dissection in one.

Gross pathology. Heart weight ranged from 275 to 610 g (mean 425). Wall thickness ranged from 1.0 to 1.6 cm (mean 1.3) for the left ventricular free wall, 1.2 to 1.7 cm (mean 1.4) for the ventricular septum and 0.20 to 0.50 cm (mean 0.35) for the right ventricular free wall.

Additional findings included combined valvular disease and grade 4 coronary artery disease in three patients, valvular disease alone in two patients and coronary artery disease alone in one patient. In only one patient with severe aortic stenosis, however, was severity of the valvular heart disease more than mild.

Histopathology. *Ventricular myocardium.* Stainable iron was exclusively sarcoplasmic; no iron was observed in the interstitium. The pattern of distribution of stainable iron was

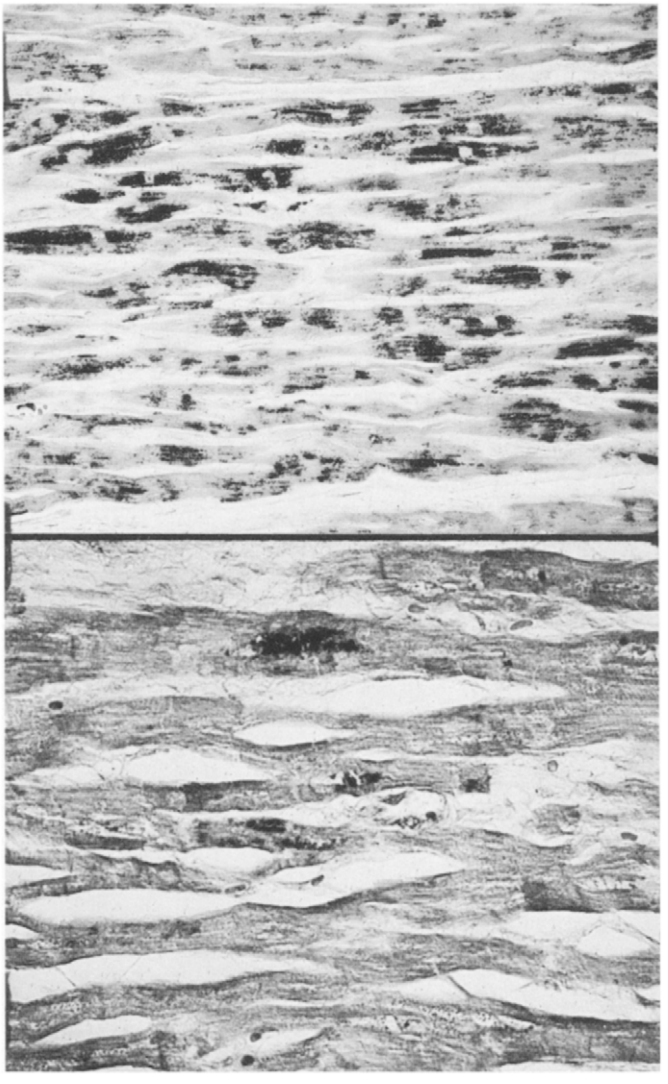


Figure 1. Stainable iron in the left ventricular myocardium of two patients. **Top,** Severe iron deposition. **Bottom,** Mild iron deposition. (Iron stain; **Top,** ×360, reduced by 15%; **Bottom,** ×180, reduced by 15%.)

either focal or diffuse, and the extent varied from none to severe (Fig. 1). Variations in extent were observed among different hearts and within individual hearts by anatomic site. In general, variations among hearts were greater than variations within a single heart.

In 10 (71%) of the 14 hearts, stainable iron was present in all ventricular sites. In one of the three hearts from patients who had undergone phlebotomy before death, there was no stainable iron at any site. Among the 11 hearts from patients without prior phlebotomy, three (27%) had no stainable iron in subendocardial specimens from the septal aspect of the right ventricle, corresponding to sites commonly used for endomyocardial biopsy in living patients (Fig. 2). In two of these three hearts, there was stainable iron at other anatomic sites. The third heart was from a patient who had had esophageal varices and a history of chronic low grade gastrointestinal bleeding that had not been treated with transfusions.

Fibrosis was either absent or mild (grade 1) at all ventricular sites from all patients.

Atrial myocardium. As in the ventricular myocardium, stainable iron was exclusively sarcoplasmic. In seven hearts (50%), stainable iron was present in atrial myocardium but the grade was less than that in the ventricles.

Conduction system. No heart had stainable iron in the sinus node. Six (43%) had stainable iron in the AV conduction system. In four of these six, stainable iron was observed in the AV node (Fig. 3) and in two hearts it was confined either to the perinodal tissues or to the bundle branches. These six hearts included the five hearts with the highest levels of ventricular elemental iron as determined by atomic absorption spectroscopy.

Iron quantitation. For all sites in the entire group with idiopathic hemochromatosis, elemental iron (Fig. 4) ranged from 433 to 7,474 μg/g dry weight (mean 1,701). Mean elemental iron for individual hearts ranged from 647 to 5,095 μg/g.

For all anatomic sites in the entire control group, elemental iron ranged from 136 to 817 μg/g (mean 399). Mean elemental iron in individual control hearts ranged from 183 to 674 μg/g.

For each anatomic site there was marked variation in the quantity of iron found in different study hearts. However, there was less variation among different anatomic sites in individual hearts. The iron levels in control hearts were lower and the range was relatively narrow.

In 3 of the 14 study hearts, elemental iron values for all anatomic sites consistently fell within the range of normal. One of these three hearts came from a patient with a history of therapeutic phlebotomy before death and one came from a patient with a history of chronic low grade gastrointestinal blood loss that had not been treated with transfusions.

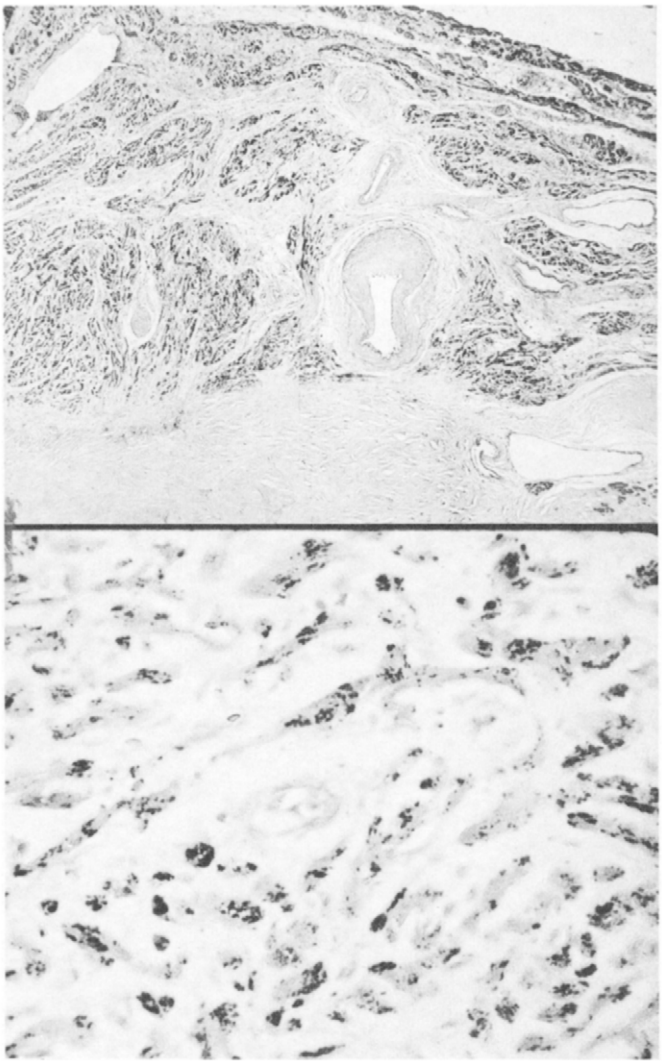
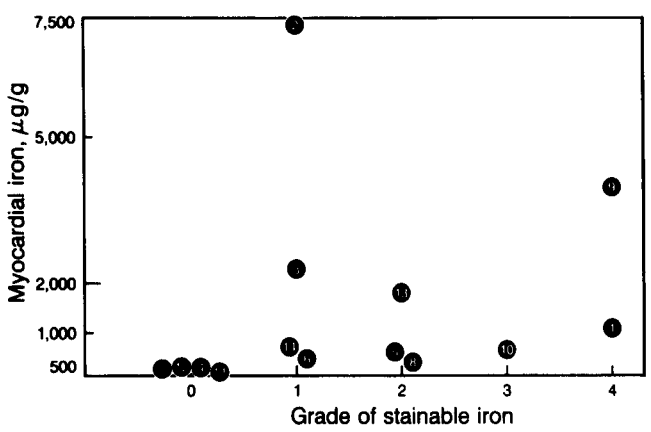


Figure 3. Stainable iron in the atrioventricular node. (Iron stain; **Top,** ×90, reduced by 15%; **Bottom,** ×360, reduced by 15%.)

Figure 2. Concentration of myocardial elemental iron (based on tissue dry weight) by histologic grade of stainable iron in the right ventricular septal subendocardium for 14 hearts from patients with idiopathic hemochromatosis. See text for grading. The **black circles** with numbers correspond to values for Patients 1 to 14.



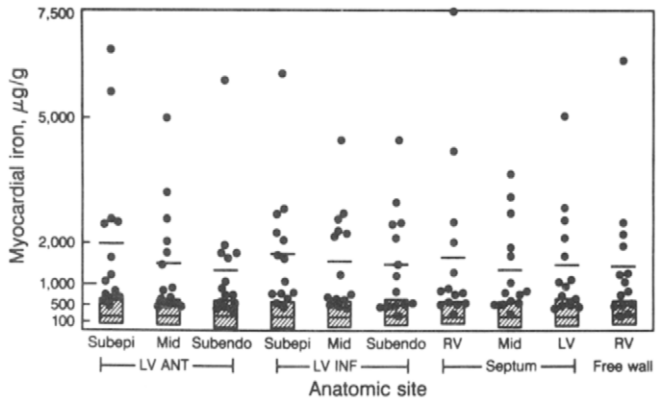


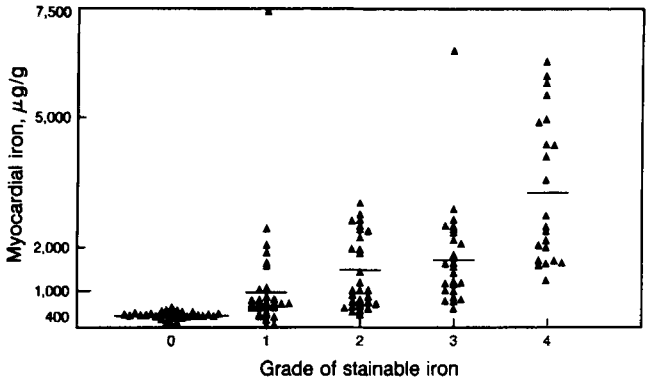
Figure 4. Concentration of myocardial elemental iron (based on tissue dry weight) determined by atomic absorption spectroscopy in hearts from idiopathic hemochromatosis patients (n = 14) and from control hearts (n = 14), displayed by anatomic site. The **black circles** correspond to iron values for hearts from the 14 idiopathic hemochromatosis patients. All values for each idiopathic hemochromatosis patient are displayed. For control patients, the range of iron values is represented for each anatomic site by a **vertical hatched bar.** Horizontal lines represent mean iron levels for each anatomic site for study and control hearts. LV ANT = left ventricular anterior wall; LV INF = left ventricular inferior wall; Mid = midmyocardial region; RV = right ventricle; Subendo = subendocardial region; Subepi = subepicardial region.

Of the 10 ventricular anatomic sites analyzed for elemental iron, the subepicardial layers had the highest levels within the study group, although there was marked individual variation. The difference between subepicardial and subendocardial sites was statistically significant ($p < 0.01$, Student-Neuman-Keuls multiple comparison procedure). No significant differences among anatomic sites were observed within the control group.

In no matched-pair analysis did the quantity of elemental iron in a control heart exceed that in a study heart at the same anatomic site. Overall, elemental iron at each of the anatomic sites was significantly higher in the hearts from patients with idiopathic hemochromatosis than in the control hearts ($p < 0.01$).

Although there appeared to be a correlation between quantitative histologic grade and median quantitative elemental iron value, the ranges of quantitative values overlapped considerably (Fig. 5).

Figure 5. Concentration (based on tissue dry weight) of myocardial elemental iron displayed by histologic grade of stainable iron for all 10 anatomic sites for each of the 14 study hearts. **Horizontal lines** represent median values of elemental iron.



Discussion

Previous studies. The association between histologically demonstrable myocardial iron and cardiac dysfunction in patients with iron overload disorders has been described (5–11). However, reports on the cardiac histopathology of iron overload disorders (5–8) have been confined almost exclusively to patients with acquired hemochromatosis associated with chronic transfusions for refractory anemias. In contrast, the histopathologic characteristics of the heart in idiopathic hemochromatosis have not been well characterized. It is unknown whether the myocardial iron distribution associated with idiopathic hemochromatosis differs from that associated with acquired hemochromatosis.

Schellhammer et al. (5) studied six hearts from autopsies of patients with acquired hemochromatosis and found stainable iron in all parts of the conduction system, including the sinus node. Vigorita and Hutchins (6) also studied cardiac histopathology in six patients with acquired hemochromatosis and reported variable grades of iron staining in different patients and among different anatomic sites. The sinus node was involved in two cases, and the AV conduction system was involved in five. Stainable iron was observed in the ventricular myocardium of all patients.

James (7) described cardiac histopathologic findings in two patients with acquired hemochromatosis and in three with idiopathic hemochromatosis. In each, the AV node contained stainable iron but the sinus node was spared. All hearts had diffuse stainable myocardial iron, and no differences were described between hearts from patients with idiopathic hemochromatosis and hearts from patients with acquired hemochromatosis.

The most comprehensive reported study of cardiac involvement in iron overload disorders, by Buja and Roberts (8), included cardiac histopathologic findings in 135 patients. However, only 19 of them had stainable iron in the myocardium; 3 of the 19 had idiopathic hemochromatosis. These authors observed that stainable iron in the cardiac conduction tissue was minimal and was less than that in atrial or ventricular myocardium. In their study, iron deposits always appeared to be greatest in the subepicardial third, intermediate in the subendocardial third and least in the middle third of the myocardium. Histopathologic differences between patients with idiopathic hemochromatosis

and those with other iron overload disorders were not described. However, two of the three patients with idiopathic hemochromatosis in their series also had received chronic transfusions for refractory anemia (one patient had leukemia and the other had hemolytic anemia). Therefore, in only one case was stainable cardiac iron attributable to idiopathic hemochromatosis alone.

Present study. In our study, the severity of cardiac iron deposition varied considerably, not only among hearts but also among anatomic sites in individual hearts. As in the report by James (7), the sinus node was spared in all of our 14 cases; the AV conduction system was involved in 6 of them. In contrast to previous studies, regional anatomic differences in ventricular myocardial iron were demonstrated by quantitative elemental analysis and by semiquantitative histologic grading of stainable iron. The subepicardium tended to contain more iron than the midmyocardial or subendocardial regions, as has been reported for patients with acquired hemochromatosis (8). Our observations regarding the variability in quantity of myocardial iron in idiopathic hemochromatosis are consistent with the variable clinical spectrum of cardiac dysfunction, ranging from asymptomatic patients with normal cardiac function to severely symptomatic patients with end-stage cardiac failure (12–14).

The subcellular distribution of stainable cardiac iron was not described in the previous studies cited. Furthermore, cardiac hemochromatosis frequently has been described as an infiltrative process, implying that iron is deposited in the interstitium. However, we observed no instances of interstitial stainable iron; iron deposition was confined exclusively to the sarcoplasm. Hence, cardiac involvement in idiopathic hemochromatosis should be regarded as a storage disease and not as an infiltrative process.

Clinical implications. In the evaluation of patients with cardiac disease of indeterminate cause, including dilated (15,16), restrictive (9) and apparent constrictive dysfunction (17), the diagnosis of idiopathic hemochromatosis should be considered. In patients who have clinical and biochemical evidence of iron overload, verification of myocardial involvement may be attempted by endomyocardial biopsy. Because stainable cardiac iron may be focal and may vary markedly by anatomic site in idiopathic hemochromatosis, multiple biopsy specimens should be obtained to minimize the possibility of sampling error in this potentially reversible form of cardiac disease.

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